Further, 4-week-old BALB/c mice were immunized with the recombinant protein in combination with Freund's complete/incomplete adjuvant according to the specific procedure. The antibody responses against JEV and WNV were determined by immunofluorescence assay and plaque reduction neutralization test (PRNT), respectively. Finally, the immunized mice were infected with lethal dose of WNV and the mortality was followed for 2 weeks.
Results: Soluble JEV envelope domain III was successfully recombinant expressed, purified and characterized. Followed by immunization with the recombinant JEV envelope domain III, high titer IgG and neutralizing antibodies against JEV were generated in mice. PRNT experiments indicated these antibodies induced by JEV envelope domain III were also capable of neutralizing WNV. Most importantly, 40% of the immunized mice survived after the lethal challenged of WNV.
Conclusion: These results for the first time demonstrated that immunization with recombinant JEV envelope domain III protein might confer partial cross-protection against WNV challenge in mice.

OL-031 The role of Interleukin-12p70 in dengue virus infection

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Background and Aim: Host resistance to dengue virus (DV) infection is dependent on both natural and acquired immune response. Following infection, the virus are able to survive and replication in a variety of nucleated cells, including nonactivated macrophages, natural killer, etc., cytokines that enhance or inhibit virus replication in these cells seem to influence the outcome of infection, as well as the pathology of the disease. Understading the role of the cytokines produced during the DV infection and their involvement in protection and pathogenesis would be essential to devise new vaccines or therapies. The aim of the study is to explore the effect of Interleukin-12p70 (IL-12p70) in the immune-pathogenesis of DV infection.
Methods: The serum levels of IL-12p70, TNF-α, IFN-γ, IL-6 and 10 were measured with ELISA in patients with dengue fever (DF). The results were compared between DF patients and normal controls, and various groups based on the course and severity of disease as well. The roles of the cytokines on pathogenesis of DV infection and disease development were explored.
Results: DF patients had significantly increased serum levels of IL-12p70, TNF-α, IFN-γ and IL-10 than those observed in controls ($p < 0.01$). The serum levels of IL-12p70, IFN-γ and TNF-α in incipient DF patients were significantly higher than those observed in convalescence DF patients ($p < 0.01$). Univariate analysis showed a similar pattern of these parameters was significantly associated with severity of the disease ($p < 0.001$). The serum IL-12p70 levels were positively correlated to serum TNF-α and IFN-γ in DF patients ($R = 0.65$, 0.72; $p < 0.001$).
Conclusion: DV infection may result in the variation of serum IL-12p70, IFN-γ, TNF-α and IL-10 levels in DF patients. Serum IL-12p70, may promote the secretion of Th1 type cytokines and inhibit the secretion of Th2 type cytokines, which play an important role in DV infection.

OL-032 Identification of miRNAs in *Aedes albopictus* and determination of their expression profiles during developmental stages and blood feeding using Solexa sequencing

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Background: miRNAs are non-coding RNAs that are recognized very important in regulating insect development and disseminating disease. *Aedes albopictus* is the most important dengue vector in China.
Methods: Total small RNAs of *Ae. albopictus* at stages of egg, larval, pupal, male adult, sugar water fed female adult, and blood meal fed female were extracted and sequenced by solexa. Northern blot was used to confirm the high-throughput sequencing results.
Results: A total of 140 *Ae. albopictus* mature miRNAs were identified, in which 91 were conserved across species whereas 49 were Aedes-specific. Clustering of miRNAs showed miRNAs specifically expressed at each developmental and blood fed female stage, as shown by six clusters in Figure 1. Northern blot showed the expression levels of miRNAs consisted with solexa sequence results.

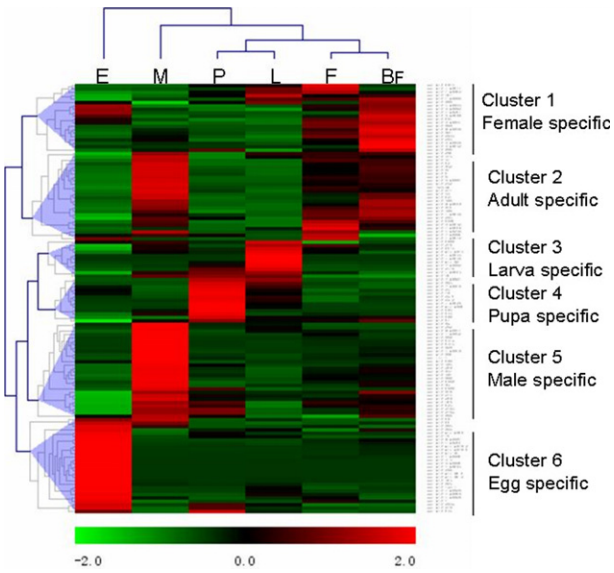

Figure 1. MiRNA expression profiles at different developmental stages by hierarchical clustering. Red indicates that a gene is highly expressed at the stage, whereas green indicates the opposite. Six clusters showed miRNAs specifically expressed at female, adult, lava, pupa male and egg stages respectively. BF, Female adult fed with blood meal; E, Egg; F, Female adult fed with sugar water; L, Larva; M, Male adult; P, Pupa.

Conclusion: The miRNAs were identified, for the first time, in *Ae. Albopictus* using a high-throughput approach. These miRNAs showed obviously different expression levels across

the 4 developmental stages in *Ae. Albopictus*, indicating their potential roles in the development.

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OL-033 The associated factors of dengue hemorrhagic fever among pediatric secondary dengue infections

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Objectives: The existence of dengue fever (DF) in Malaysia was first described following an outbreak in 1902 and the first case of dengue hemorrhagic fever (DHF) was reported in 1962. An annual incidence is about 400 to 7,000 cases. DHF is a common phenomenon in both adults and children. The aim of the study was to describe the associated factors of DHF in pediatric patients.

Methods: We reviewed all pediatric cases of laboratory confirmed secondary dengue infections from January 2005 to December 2006. The cases were selected based on serological test on the presence of dengue specific IgG and the age less than 16 year old. The data collection was made with regards to the patient's demography, clinical presentation and laboratory profiles. Pearson Chi-square, Fisher's exact, independent t-test and Mann-Whitney's test were used accordingly to study the association.

Results: Thirty-eight cases of pediatric secondary DF were identified during study period. Seven (18.4%) DHF I and two (5.3%) DHF II cases were identified according to WHO classification. The mean age was 12.4 + 3.7 year-old, 55.3% male, 94.7% Malay, 7.9% imported cases, 50.0% have history of contact with dengue patients and the mean fever days before admission was 5.0 + 1.6 days. DHF was associated with hepatomegaly ($p = 0.040$); ascites or pleural effusion ($p = 0.012$); longer hospital stay ($p = 0.012$); lower platelet during admission ($p < 0.001$) and higher alkaline phosphatase (0.015). There was no significant association between DHF with any presenting symptoms. No patient needs intensive care management. There was also no significant difference in term of outcome of infection between DF and DHF. No patient died in this series.

Conclusion: With this limited number of patients, pediatric DHF is significantly associated with hepatomegaly and elevated liver enzyme suggesting hepatitis may play important role in the pathogenesis of DHF.

OL-034 Association between La Niña and dengue fever cases in Lahore from 2005 to 2008

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Objective: To determine the association of macroclimatic and microclimatic variations and dengue fever cases admitted in various hospitals of Lahore.

Methods: Record of confirmed dengue fever cases admitted in various hospitals in Lahore was collected. Microclimatic data was obtained from Regional Meteorological Centre, Lahore which included rainfall (mm/month), humidity (% at 8 am and 5 pm) and temperature (°C). Macroclimatic data including SOI, ONI and El Niño/La Niña updates were obtained from World Meteorological Organization and National Oceanic & Atmospheric Administration, USA.

Results: There were a total of 1757 indigenously confirmed cases of dengue fever in Lahore from 2005 to 2008. Linear regression models found statistically significant correlation between SOI and minimum temperature ($r = -0.76$, $p < 0.05$) as well as maximum temperature ($r = -0.678$, $p < 0.05$) but no correlation between SOI and rainfall in Lahore ($r = -0.029$, $p > 0.05$). However, there was positive correlation between daily rainfall and ONI ($r = -0.946$, $p < 0.05$). highest number of dengue fever cases were recorded in 2008 which had a positive correlation with La Niña (90% higher than the mean number of cases) when compared with years having weak or no La Niña in which case there was a reduction in number of cases compared to mean value ($p < 0.001$). A positive correlation was found between SOI and dengue ($r = 0.781$, $p < 0.05$). Dengue fever cases in Lahore were positively correlated with minimum temperature ($r = -0.967$, $F = 61.84$, $p < 0.05$), maximum temperature ($r = 0.981$, $F = 52.1$, $p < 0.05$) and rainfall ($r = 0.543$, $p < 0.05$). No significant correlation was found between relative humidity and dengue fever cases ($p > 0.05$).

Conclusion: The results show that global climate anomalies and regional weather indicators affected by it have an influence on number of patients admitted with dengue fever hospitals in Lahore. It is concluded that dengue fever epidemic can be predicted by oceanographic and meteorological data.

OL-035 Does absence of itch-response to mosquito bite enhance susceptibility to the chikungunya virus infection?

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Background: Itch response to mosquito bite is an immune response against foreign proteins injected with mosquito bite. We observed a substantially large segment of chikungunya patients reporting for the lack of itch response to mosquito bite. Hence we studied the recent chinkungunya epidemic (2006–2009) to explore the possible relation if any between the absence of itch response and an individual's susceptibility to chikungunya.

Method: We retrospectively studied the itch sensitivity patterns in 3000 subjects confirmed serologically for the chikungunya virus infection. The findings were compared with another set of 3000 subjects matched for age and sex, belonging to the same socioeconomic class and residing in the same areas presenting with other diseases with no current or past history of chikungunya virus infection as per the National Institute of Communicable diseases (NICD), India case definition. Data analysis was conducted using software GraphPad instat.

Results: The Odds of acquiring the chikungunya infection in patients without the history of itch response to mosquito bites is much higher compared (OR: 4.880, 95% CI: 4.373–5.444) to those with strong history of itch response demonstrated by itching and wheal reaction. Among the subjects diagnosed clinically for chikungunya it was also observed that the disease symptoms like joint pain (OR: 5.406, CI: 4.319–6.767), retro orbital pain (OR: 2.299, CI: 1.936–2.730), were more pronounced ($P < 0.0001$) in those with absence of itch response compared to those with positive history of itch response. Symptom of myalgia